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PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'VII) (P) '
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'VII) (P) '
L1            9 (OMPT PROTEASE OR PROTEASE VII) (P) (ACTH OR MOTILIN OR (CALCITO
                NIN PRECURSOR))

=> S ((ompt protease) or (protease VII)) (P) (acth or motilin or (calcitonin
precursor))
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED ') (P) '
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED ') (P) '
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L2            9 ((OMPT PROTEASE) OR (PROTEASE VII)) (P) (ACTH OR MOTILIN OR (CAL
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L3             2 DUPLICATE REMOVE L2 (7 DUPLICATES REMOVED)

=> d l3 1-2 bib ab

L3   ANSWER 1 OF 2  HCAPLUS  COPYRIGHT 2009 ACS on STN DUPLICATE 1
AN   2005:300594  HCAPLUS
DN   142:368184
TI   Production of biol. active polypeptides by the proteolysis of recombinant
     synthetic polypeptide precursors by the OmpT protease variants
IN   Okuno, Kazuaki; Yabuta, Masayuki
PA   Daiichi Suntory Pharma Co., Ltd., Japan
SO   PCT Int. Appl., 107 pp.
     CODEN: PIXXD2
DT   Patent
LA   Japanese
FAN.CNT 1
     PATENT NO.          KIND   DATE        APPLICATION NO.        DATE
     --------------      ----   --------    -------------------    --------
PI   WO 2005030956        A1    20050407    WO 2004-JP14704        20040929
         W:  AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH,
             CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD,
             GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC,
             LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI,
             NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY,
             TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW
         RW: BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM,
             AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK,
             EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE,
             SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE,
             SN, TD, TG
     AU 2004276687        A1    20050407    AU 2004-276687         20040929
     CA 2540446           A1    20050407    CA 2004-2540446        20040929
     EP 1674567           A1    20060628    EP 2004-773628         20040929
         R:  AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,
             IE, SI, FI, RO, CY, TR, BG, CZ, EE, HU, PL, SK
     BR 2004014611        A     20061107    BR 2004-14611          20040929
     CN 1860226           A     20061108    CN 2004-80028525       20040929
     KR 2006089724        A     20060809    KR 2006-705984         20060327
     US 20070077617       A1    20070405    US 2006-573821         20060328
PRAI JP 2003-342183       A     20030930
     WO 2004-JP14704      W     20040929
ASSIGNMENT HISTORY FOR US PATENT AVAILABLE IN LSUS DISPLAY FORMAT
AB   The proteolytic method for producing biol. active polypeptides (
     ACTH (1-24), motilin or calcitonin) from recombinant
     synthetic precursor polypeptides or fusion proteins by using OmpT
     protease mutants has been developed.  The synthetic precursor
     polypeptides or fusion proteins (22 .apprx. 45 a.a. (amino acid)) have
     been designed according to the substrate specificities of the OmpT
     protease mutants.  Synthetic substrate polypeptides have Arg or
     Lys at P1 site and the a.a. other than Asp, Glu or Pro at the P1' site.
     The substrate polypeptides have one, two or serial three basic a.a. in the
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P10 .apprx. P3, P10 .apprx. P3' or P10 .apprx. P5' (more specifically in the P5 .apprx. P3 site), however the sites P6 and P4 are excluded if only one basic a.a. in the sequence. The fusion protein substrates with protection peptide having C-terminal Arg or Lys have N-terminal a.a. such as Phe, Ala, Ser, Cys or Tyr and the other a.a. excluding Asp, Glu and Pro. These preferred P5 .apprx. P1 sequence and P7 .apprx. P1 sequence in the synthetic precursor polypeptides or fusion proteins are Arg-Arg-Arg-Ala-Arg and Asp-Ala-Arg-Arg-Arg-Ala-Arg, resp. Introduction of acidic a.a. typically Asp to the P3 site can repress the digestion by the OmpT proteases. The OmpT protease variants that can be used in the proteolysis system have a.a variation at the 97th position. The 97th a.a. is Leu, Met or His and the other a.a. including Ala, Phe, Ser, Thr, Cys, Asn, Gln, and Glu. The vector encoding the fusion substrate protein containing human glucagon, motilin, ACTH or calcitonin was designed to satisfy the structural condition claimed above and expressed in the inclusion body of E. coli and the cleaving of biol. active peptides from the substrate fusion proteins by the recombinant OmpT protease variant was demonstrated. The performance of the coexpression system of the substrate fusion protein and OmpT protease variant in the biol. active peptide generation was also demonstrated.

RE.CNT 5 THERE ARE 5 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 2 OF 2 MEDLINE on STN DUPLICATE 2
AN 2004028684 MEDLINE
DN PubMed ID: 14711628
TI Utilization of Escherichia coli outer-membrane endoprotease OmpT variants as processing enzymes for production of peptides from designer fusion proteins.
AU Okuno Kazuaki; Yabuta Masayuki; Ooi Toshihiko; Kinoshita Shinichi
CS Institute for Medicinal Research and Development, Daiichi Suntory Pharma Co., Ltd., Akaiwa, Chiyoda-machi, Ohra-gun, Gunma 370-0503, Japan.. Kazuaki_Okuno@dsup.co.jp
SO Applied and environmental microbiology, (2004 Jan) Vol. 70, No. 1, pp. 76-86.
Journal code: 7605801. ISSN: 0099-2240.
Report No.: NLM-PMC321264.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200404
ED Entered STN: 21 Jan 2004
Last Updated on STN: 9 Apr 2004
Entered Medline: 8 Apr 2004
AB Escherichia coli outer-membrane endoprotease OmpT has suitable properties for processing fusion proteins to produce peptides and proteins. However, utilization of this protease for such production has been restricted due to its generally low cleavage efficiency at Arg (or Lys)-Xaa, where Xaa is a nonbasic N-terminal amino acid of a target polypeptide. The objective of this study was to generate a specific and efficient OmpT protease and to utilize it as a processing enzyme for producing various peptides and proteins by converting its substrate specificity. Since OmpT Asp(97) is proposed to interact with the P1' amino acid of its substrates, OmpT variants with variations at Asp(97) were constructed by replacing this amino acid with 19 natural amino acids to alter the cleavage specificity at Arg (P1)-Xaa (P1'). The variant OmpT that had a methionine at this position, but not the wild-type OmpT, efficiently cleaved a fusion protein containing the amino acid sequence -Arg-Arg-Arg-Ala-Arg downward arrow motilin, in which

motilin is a model peptide with a phenylalanine at the N terminus. The OmpT variants with leucine and histidine at position 97 were useful in releasing human adrenocorticotropic hormone (1-24) (serine at the N terminus) and human calcitonin precursor (cysteine at the N terminus), respectively, from fusion proteins. Motilin was produced by this method and was purified up to 99.0% by two chromatographic steps; the yield was 160 mg/liter of culture. Our novel method in which the OmpT variants are used could be employed for production of various peptides and proteins.